Anthropogenic impacts on life history traits on Eastern Chipmunks *(Tamias striatus)*

by

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General Abstract

Urban centers provide unique habitats for opportunistic animal species. However, conditions in urban habitats are inherently stressful and require specialized adaptations in resident animals. Eastern chipmunks are a generalist small rodent species common in a number of different habitat types. I examined differences in behavioural traits and stress levels (chapter one), as well as body condition and parasitism rates (chapter 2) between urban and natural populations of chipmunks. I tested the hypothesis that 1), chipmunks in urban habitats would show greater levels of boldness and exploratory behaviour, 2), urban chipmunks would show greater levels of stress reflected in greater cortisol concentrations in hair and feces, 3) urban chipmunks would have a relatively poor body condition due to poor quality food and competition in urban centers, and 4) urban chipmunks would show greater parasitism rates measured in greater oocyte prevalence, richness, and abundance from fecal samples. A total of 140 individual chipmunks (80 natural and 60 urban) were sampled from natural habitats in Algonquin Provincial Park and outside of Sudbury, and urban habitats in Huntsville and Sudbury Ontario. Differences in behavioural patterns suggest urban animals may be more habituated to human presence, while body condition was significantly superior in urban animals. However, cortisol measured from hair and feces, leukocyte concentrations measured from blood and parasitism impacts did not differ between individuals from the two habitat type. Overall, chipmunks in these urban habitats have a better body condition and are more habituated to human presence than their natural conspecifics.

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Chapter 2

General Introduction

Rapid expansion and development of human infrastructure creates pressure for animal species (Stockwell *et al.*, 2003, Blumstein *et al.*, 2005, Harris *et al.*, 2013). An urban habitat is defined as downtown centers, suburbia, and the urban rural fringe of cities, towns, and villages, where non-domesticated animals come into regular contact with humans and human activities (Adams 2005). The development of dense human settlements often drives away or extirpates indigenous populations (Marzluff *et al.*, 2001). These anthropogenic alterations to a landscape can impose rapid evolutionary change within a few short generations, and can create expressed traits in individuals that differ from those in individuals that did not experience urbanization. (Stockwell *et al.*, 2003, Adams 2005).

Urban habitats can differ greatly from natural habitats. Rates of predation risk, climate, food resources, and competition in urban centers can increase or decrease relative to surrounding natural habitats (Miller *et al.*, 2003, Liker *et al.* 2008, Møller 2009, Sih *et al.*, 2011, Bonier 2012). Urban centers fragment habitats, destroy or modify the landscape and create novel selective pressures that can generate rapid evolutionary change in resident animal species (Stockwell *et al.*, 2003, Adams 2005, Sih *et al.*, 2011, Harris *et al.*, 2013). These human created habitats can increase edge effects, local extinctions, and reduce areas of suitable habitat for individuals (Nupp and Swihart 1998, Fernandez-Juricic 2000, Stockwell *et al.*, 2003, Adams 2005, McGregor *et al.*, 2008, Harris *et al.*, 2013). This causes changes at the community level, such as decreases in species richness as urbanization increases, and can increase biomass and population density of remaining species (Nupp and Swihart 1998, Stockwell *et al.*, 2003, Adams 2005, Shochat *et al.*, 2006, Liker *et al.*, 2008, Møller 2009, Harris *et al.*, 2013).

The novel environment created by urban centers facilitates development of specialized traits within urban populations (Marzluff *et al.*, 2001). Animals that are most likely to survive in

fragmented urban habitats are those that thrive on edge effects, are versatile and adaptable, and have a broad habitat tolerance (Nupp and Swihart 1998, Miller *et al.*, 2003, Adams 2005, Møller 2009, Sih *et al.*, 2011, Harris *et al.*, 2013). Urbanization also favours residency over migration, a diverse diet (i.e. omnivores) and habituation to human interactions (Møller 2009, Powell *et al.*, 2013).

Many of the conditions experienced in urban centers affect the energetic demands on individual animals living there. Humans are perceived as a potential predator by many species (Gill *et al.*, 1996, Fernandez-Juricic 2000, Marzluff *et al.*, 2001, Frid and Dill 2002, Stillman and Goss-Custard 2002, Møller 2009, Powell *et al.*, 2013). Human activities such as tourism, recreation, and industrial development impose increased energetic demands on wildlife (Gill *et al.*, 1996, Møller 2009). These human activities have been shown to drastically decrease foraging times and food consumed, and can lead to a decrease in overall fitness (Gill *et al.*, 1996, Fernandez-Juricic 2000, Blumstein *et al.*, 2005, Martin and Réale 2008). Furthermore, human activities can alter breeding periods, often extending or causing earlier breeding seasons than in natural areas (Fernandez-Juricic 2000, Ditchoff *et al.*, 2006, Martin and Réale 2008). Urban animals can also suffer stress and mortality from roads and traffic (Ditchoff *et al.*, 2006, McGregor *et al.*, 2008, Evans *et al.*, 2010, Powell *et al.*, 2013). Increases in predation may occur due to the presence of domesticated cats and dogs; however, predation rates may also decrease due to the absence of predators from other non-urban habitats. Finally, urban animals are exposed to a variety of other unique stressors such as light, noise, and chemical pollution (Evans *et al.*, 2010, Sih *et al.*, 2011).

Increases in production of hormones such as corticosterone and cortisol can be associated with higher rates of energetic requirements in animals (Kenagy and Place 2000). Baseline corticosterone levels correlate with an animal's overall body condition, and are also indicative of other factors such as food abundance and quality, habitat, and dominance status (Kitaysky *et al.*, 1999, Bonier *et al.*, 2007). Increases in stress hormone production may be costly on an organism's

energy reserves and may reduce resources organisms have for other functions, such as growth, reproduction, and maintenance of hemostasis. (Boissy 1995, Sheldon and Verhulst 1996, Kitaysky *et al.*, 1999, Duckworth *et al.*, 2001).

Animals with a specific set of behavioural traits are more likely to persist in urban habitats. Behavioural traits are either inherited or learned behavioural patterns in individuals that generally remain consistent over a variety of situations (Verbeek *et al.*, 1996, Dingemanse and Réale 2005, van Ores *et al.*, 2005, Boon *et al.*, 2007, Stamps 2007). Such traits can show great degrees of variation even between individuals in the same population. Behavioural traits are found in a variety of taxa including mammals, birds, fish, amphibians, reptiles, arthropods, and molluscs (Sih *et al.*, 2004, Bell 2007). Five behavioural traits that are typically measured include boldness/shyness, exploratory, activity, aggressiveness, and sociability (Drent *et al.*, 2003, van Ores *et al.*, 2004, Réale *et al.*, 2007, Biro and Stamps 2008).

An organism's behavioural response is important in determining its success or failure when exposed to new or changing environmental conditions (Sih *et al.*, 2011). Personality, defined as a suite of individual characteristics that describe and predict patterns in feeding and behaviour, has been shown to be correlated with dominance, natal dispersal, territorial quality and reproductive success, survival, physiological responses to stress, and offspring recruitment (Verbeek *et al.*, 1996, Réale *et al.*, 2007). For instance, bold big horn ewes are less likely to be predated by cougars than less bold individuals (Réale and Festa-Bianchet 2003, Boon *et al.*, 2007). Similarly, low sociability, high aggressiveness, and more risk taking behaviours are associated with reduced parental investment in many species including red squirrels *Tamiasciurus hudsonicus* (Boon *et al.*, 2007, Réale *et al.*, 2007).

Urban conditions facilitate the expression of particular sets of behavioural phenotypes. Animals with specific behavioural traits, such as boldness and exploratory behaviour, are likely to

locate and settle in urban areas (Réale and Festa-Bianchet 2003, Møller 2009, Evans *et al.,* 2010). Studies of urban animals show they are often uncharacteristically bold in the face of humans, perhaps as a result of habituation and/or the advantages of boldness in colonizing and adapting to a novel environment (Marzluff *et al.,* 2001, Martin and Réale 2008, Møller 2009, Evans *et al.,* 2010). For example, boldness was significantly higher in urban song sparrows (*Melospiza melodia*) compared to natural conspecifics, and boldness did not differ between two separate natural populations (Evans *et al.,* 2010).

Urban conditions can also significantly influence the health of individual animals living in urban populations. Body condition, defined as the energetic state of an animal (Schulte-Hostedde *et al.,* 2005) is associated with many behavioural, physiological, and life history traits in animal populations (Blumstein *et al.,* 2005). In natural settings, a good body condition is especially adaptive, especially when food resources are low (Schulte-Hostedde *et al.,* 2005, Liker *et al.,* 2008). However, in urban settings, food availability from anthropogenic sources is generally sufficient to sustain even individuals with a poor body condition, as the presence of such resources is generally reliable year around (Shochat 2004, Liker *et al.,* 2008). One possibility is that opportunistic individuals are able to take advantage of the abundant food resources and consume far more than what is necessary for survival. This type of behaviour limits food availability for the less opportunistic individuals, creating a population with high numbers of individuals with relatively poor body condition (Shochat 2004, Liker *et al.,* 2008). Body condition in urban centers is also influenced by high levels of toxic chemicals and metals from anthropogenic food and waste sources (Shochat 2004, Liker *et al.,* 2008, Sih *et al.,* 2011). Many anthropogenic food sources have poor quality and are insufficient for growth and development in juvenile animals (Shochat 2004). For instance, juvenile gulls fed food from anthropogenic sources are noted to have lower fledging rates than those fed fish caught at sea (Shochat 2004).

High population densities, such as those found in urban centers, can facilitate parasite and disease transmission. Parasites increase their own survival with a variety of immunosuppressant strategies within their host species (Barnard *et al.,* 1998). Secondary effects of parasitism can impede growth and reproduction either directly or indirectly via resource allocation, which is energetically costly to the host (Neuhaus 2003, Hillegass *et al.,* 2010, Gooderham and Schulte-Hostedde 2011, Harris *et al.,* 2013). Parasitism also decreases attractiveness to a potential mate (Patterson and Schulte-Hostedde 2011). Poor nutrition, as experienced in urban populations is associated with a decrease in immune function, and thus, an increase of susceptibility to parasitism (Sheldon and Verhulst 1996). For instance, a negative relationship in reproductive success was found in red squirrels with a high abundance of endoparasites (Gooderham and Schulte-Hostedde 2011) where reproductive success was notably higher in female cape ground squirrels when treated for ectoparasites and endoparasites (Hillegass *et al.,* 2010).

The eastern chipmunk, *Tamias striatus*

The eastern chipmunk, *Tamias striatus,* is a small rodent species native to much of eastern and central Canada and the United States. Chipmunks typically display agonistic behaviour towards one another and are highly territorial (Blair 1943). Chipmunks breed in the summer months, between March-April, and June-July, and can have up to two annual litters with one to six offspring each (Yerger 1955). Juveniles emerge from their burrows after one month (Yerger 1955). Females have been noted to reach sexual maturity between 3-7 months, although both sexes typically do not breed until the following year (Yerger 1955). Chipmunks have two moulting periods, in early summer and late fall (Yerger 1955, Martin and Réale 2008). Chipmunks consume a wide variety of food in their diets, including berries, seeds, invertebrates, and even small vertebrates including frogs; food availability seems to be the largest factor when taking dietary choices into consideration (Yerger 1955). Chipmunks store food in caches for use in the winter months (Yerger 1955).

Chipmunks make an ideal candidate for a study comparing urban and natural effects, as they are easy to trap because of their small size, territoriality, and abundance in both urban and natural environments. They have been used in a wide variety of studies to assess behavioural changes to environmental stimuli (Martin and Reale 2008, Patterson and Schulte-Hostedde 2011). Chipmunk populations decline as a response to habitat fragmentation (Nupp and Swihart 1998), suggesting that clear differences may exist between urban and natural populations. Although chipmunks are not a threatened species, they can serve as a model species for species threatened by human activities. Many of the patterns and trends shown in urban mammals reflect trends in birds, amphibians, reptiles, and invertebrates (Adams 2005). Increases in human expansion reduce the habitat available to wildlife and force animals to come in more frequent contact with human activities (Stockwell *et al.*, 2003). Studying the effects of long term urbanization in a common species can help predict the effects on endangered ones (Blumstein *et al.*, 2005). Threatened species that come into frequent contact with human beings can be assessed against these standards and predictions can be made as to their success or failure at human co-habitation.

Project overview

The goal of this project was to use *T. striatus* as a model species to answer several research questions based around the premise that long term urbanization causes various changes in urban mammal populations when compared to their natural counterparts. Specifically, I predicted behavioural traits displayed by individuals in urban settings would differ significantly from those displayed by individuals in non-urban habitats. Similarly, I predicted that the types of stressors experienced in urban centers caused by the abundance of humans and contact with human activity would lead to differences in physiology between urban and natural populations of chipmunks. Finally, I predicted that the various pressures experienced in urban environments by resident urban

animals would result in differences in body condition and parasitism rates between urban and natural populations.

In order to answer the specific questions of this research, I have organized the thesis in two chapters. In chapter 1, I focused on the behavioural and hormonal differences between urban and natural populations of chipmunks. I used a novel environment as a method to determine behavioural syndromes displayed by the studied animals. Because boldness and activity may be adaptive for dealing with stress and human interaction, I predicted higher rates of bold and aggressive behaviours in urban individuals (Evans *et al.,* 2010). I also predicted greater levels of exploratory behaviour in urban populations, since exploratory behaviour is adaptive for animals colonizing urban centers (Møller 2009). Hair and fecal samples were used to measure stress response via concentrations of cortisol. Measuring adrenal glucocorticoid hormones, such as cortisol, is an effective method for measuring stress responses and adrenocortical activity in mammals (Davis *et al.,* 2008, Macbeth *et al.,* 2010, Ashley *et al.,* 2011, Dettmer *et al.,* 2012). Hair and feces accumulate glucocorticosteroids over differing time periods, and sampling hair and faeces is a non-invasive method to measure them without the risk of spiking glucocorticosteroid levels in plasma, that may arise from the stress caused by handling (Ashley *et al.,* 2011). I predicted higher cortisol concentrations in hair and fecal samples taken from urban chipmunk populations, as high cortisol levels are associated with stress, and human contact is inherently stressful in most species (Møller 2009). Alternatively, due to a greater food abundance and a lack of non-urban predators, cortisol concentrations may be lower in urban animals compared to their non-urban conspecifics. I also performed leukocyte counts from blood samples collected from urban and natural chipmunks. Under stressful circumstances, concentrations of leukocytes, as well as the ratio of neutrophils to lymphocytes in mammalian blood, typically increase (Davis *et al.,* 2008). I thus predicted that due to the stressful circumstances of

living in urban environments, ratios of neutrophils to lymphocytes and total leukocyte counts would be significantly higher in urban animals compared to natural ones.

In chapter 2, I assessed the effect of urbanization on body condition (overall body mass and size), and health (using parasite load as a proxy) of chipmunks living in urban centers by comparing them to individuals living in natural environments. The average body condition of urban animals was expected to be lower as compared to natural individuals, because overexploitation of food may reduce foraging success of less competitive individuals, or because urban food quality is generally expected to be inferior (Liker *et al.,* 2008, Møller 2009). The rate of endoparasite infection (i.e. parasites that live within their host's body, typically in the intestine) was measured by the number different types of oocytes identified (richness), and totals of parasite eggs estimated in fecal samples, as well as the prevalence of each parasite type. Because urban conditions tend to be isolated from non-urban habitats, and facilitate high densities of many urban species, as well as high rates of disease transmission (Møller 2009), I expected to see greater presence of endoparasites via endoparasite oocyte richness, prevalence, and abundance in urban samples. I also looked at the effects of parasitism using the percentage of eosinophils in leukocytes; eosinophil concentrations normally increase in response to high endoparasitism; so, I predicted that eosinophils percentages would be higher in urban populations (Nunn *et al.*, 2000, Dhabhar 2002, Davis *et al.*, 2008).

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Chapter 1

Effects of urbanization on stress and behaviour in eastern chipmunks (*Tamias striatus*)

Abstract

Urban habitats and human interactions can be challenging for resident animal and facilitate the reflection of different behavioural expressions than those in non-urban habitats. I tested the hypothesis that these beneficial adaptations would be reflected in urban eastern chipmunks (*Tamias striatus)* and that these traits would differ from those reflected in their natural conspecifics. Furthermore, I predicted that greater stress levels in urban settings would appear as increased cortisol concentrations and increased leukocyte totals and neutrophil/lymphocyte ratios when compared with those taken from natural animals. I used Huntsville and Sudbury, Ontario for my urban sites and Algonquin Provincial Park and areas outside Sudbury for my natural sites respectively. I live trapped chipmunks and tested behavioural responses to a novel environment with an open field trial as well as docility and habituation with a handling bag test. I collected hair and fecal samples as a measure of cortisol concentration and examined blood smears to assess leukocyte types and abundance. My findings did show significant differences between behavioural variables expressed, specifically greater locomotion (activity) in natural habitats and more rearing (exploratory) in urban animals. A significant difference in higher fecal cortisol was detected in animals from natural habits but no difference was detected in hair cortisol samples. Finally no differences were detected in leukocyte totals or neutrophil/lymphocyte ratios assessed from blood smears between urban and natural chipmunk populations. These results suggest that urban conditions are less challenging than non-urban ones, though perhaps chipmunks were able to move between habitats.

Introduction

For animals, an urban habitat is defined as downtown centers, suburbia, and the urban-rural fringe of cities, towns, and villages, where non-domesticated animals come into regular contact with humans and human activities (Adams 2005). Urban areas expose animals to a variety of stressors not encountered in more natural environments. Stress is defined as how an organism responds physiologically, psychologically, and behaviourally to an external stimulus that challenges or threatens its wellbeing and exceeds the individuals' resources for dealing with the stimulus (Ulrich *et al.*, 1991, Morgan and Tromborg 2007). Human activity and interactions with humans are a primary source of stress to urban animals (Marzluff *et al.*, 2001, Frid and Dill 2002, Stillman and Goss-Custard 2002, Møller 2009, Ashley *et al.*, 2011, Powell *et al.*, 2013). Additional urban situations can be considered stressors such as traffic, light, noise, and chemical pollution, as well as mortality risk from domesticated animals and road crossings (Ditchoff *et al.*, 2006, McGregor *et al.*, 2008, Evans *et al.*, 2010, Sih *et al.*, 2011, Powell *et al.*, 2013). Urban animals live in high densities that can also lead to density dependant selective pressures on male-male competition, immunology, and intraspecific competition for resources (Ditchoff *et al.*, 2006, Mennechez and Clergeau 2006, Møller 2009, Harris *et al.*, 2013, Powell *et al.*, 2013). Urban animals are often isolated from other populations by natural and anthropogenic barriers which have the potential to limit gene flow and create genetic drift (McGregor *et al.*, 2008). These impacts can be inherently challenging to urban animal populations and can require plasticity in behavioural responses in order for animals to adapt (Atwell *et al.*, 2012).

Urban Stressors

In animals, the stress response is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which mediates production and release of hormones such as corticosterone and cortisol (Kenagy and Place 2000, Macbeth *et al.*, 2010, Ashley *et al.*, 2011, Bonier 2012). These hormones are involved in

the individuals' general homeostasis control as well as preparing the body for handling acute and chronic stress responses (Kenagy and Place 2000). Baseline cortisol levels can correlate with an animal's overall body condition, and are also indicative of other factors such as food abundance and quality, habitat, life history stage, and dominance status (Kitaysky *et al*., 1999, Bonier *et al*., 2007). While stress hormone production assists short term survival benefits (i.e. flight or fight response) (Dhabhar 2009); production of stress hormones over a long period of time may limit the amount of resources animals may have for other functions, such as immune function, growth, and reproduction (Boissy 1995, Sheldon and Verhulst 1996, Kitaysky *et al.,* 1999, Koolhaas *et al.,* 1999, Duckworth *et al.,* 2001).

The effects of exposure to short and long term stressors can also be measured by leukocyte type and ratios. Five types of leukocytes are found in most vertebrate taxa (lymphocytes, monocytes, neutrophils, basophils, and eosinophils) and each leukocyte type has a specific function (Davis *et al.,* 2008). Neutrophils are the most abundant type and are associated with infection, inflammation, and increases in stress (Davis *et al.,* 2004, Davis *et al.,* 2008). Increased stress hormone production can cause measurable increases in leukocyte abundance, as well as increased neutrophil production and decreased lymphocyte counts, known as the neutrophil/lymphocyte ratio (Nunn *et al.,* 2000, Dhabhar 2002, Davis *et al.,* 2008). These ratios are used in studies of mammals to compare stress levels among populations (Nunn *et al.,* 2000).

Behavioural adaptations to stress response

Behavioural syndromes are found in a variety of taxa including mammals, birds, fish, amphibians, reptiles, arthropods, and molluscs (Sih *et al.,* 2004, Bell 2007) and refer to a series of correlated traits in individuals that remain consistent over a variety of situations (Verbeek *et al.,* 1996, Dingemanse and Réale 2005, van Ores *et al.,* 2005, Boon *et al.,* 2007, Stamps 2007, Dammhahn 2012). Some of the behavioural syndromes identified in studied species include

boldness/shyness, exploratory, activity, aggressiveness, and sociability (Drent *et al.,* 2003, van Ores *et al.,* 2004, Réale *et al.,* 2007, Biro and Stamps 2008). Behavioural traits are hereditable and can differ in individuals within the same population (Dall *et al.,* 2004). Heritability of behavioural syndromes may explain why some behavioural traits appear maladaptive, such as pre-copulation mate eating in spiders, and risky feeding behaviours in sticklebacks (Bell 2007, Evans *et al.,* 2010).

Certain behavioural traits are correlated with the unique habitat conditions found in urban centers. Animals with specific behavioural traits, such as boldness, docility and exploratory behaviour, are more likely to explore and settle in new habitats and are thus more likely to settle in urban areas (Møller 2009, Evans *et al.,* 2010). Similarly, docility and exploratory behaviour increase with increased habituation to human activities (Martin and Réale 2008 a). In a study by Evans *et al.,* (2010), boldness was significantly higher in urban song sparrows (*Melospiza melodia*) as compared to those living in more natural habitats, and boldness did not differ between two separate natural populations. Greater expression of bold behaviour in urban animals have been correlated with a reduced response to predation risk (Boissy 1995, Biro and Stamps 2008, Møller 2009, Evans *et al.,* 2010). Docility and habituation to "normal" levels of human presence were noted as being greater in urban chipmunks where compared to their natural conspecifics (Bateman and Flemming 2014).

Study Species

The eastern chipmunk, *Tamias striatus,* is a small rodent native to much of eastern central Canada and the United States. They have wide home ranges that show overlap between males, females, and juveniles (Blair 1943). They have been used in a wide variety of studies to assess behavioural changes to environmental stimuli (Martin *et al.,* 2011, Patterson and Schulte-Hostedde 2011). Moreover, results from previous chipmunk studies have shown a high degree of repeatability

and chipmunks are relatively easy to recapture for studies involving multiple trials, making them useful as a model species (Yerger 1955, Martin *et al.*, 2011, Patterson and Schulte-Hostedde 2011).

The purpose of this study was to use *T. striatus* as a model species to test the hypothesis that urban environments are more stressful than natural environments for animals, and that there would be significant differences in behavioural syndromes and stress levels in urban animals as compared to animals from natural settings. Because boldness and aggressiveness are adaptive for dealing with stress and human interaction (Evans *et al.*, 2010), I predicted higher rates of boldness to be observed in urban individuals. I also predicted greater levels of exploratory behaviour in urban populations, because exploratory behaviour is adaptive for animals colonizing urban centers (Møller 2009). I also expect to Since high cortisol levels are associated with stress, and human contact is inherently stressful to most non-domesticated species (Møller 2009, Evans *et al.*, 2010, Bonier 2012), I predicted higher cortisol concentrations in urban chipmunk populations as compared to natural ones. Similarly, I expected that due to the stressful conditions of living in urban environments, leukocyte counts should be significantly higher in urban chipmunks. As higher concentrations of neutrophils and lower concentrations of lymphocytes are associated with higher levels of chronic stress (Davis *et al.*, 2008), I predicted a significant increase in the neutrophil/lymphocyte ratio in urban populations compared to natural ones. While many similar studies have been performed comparing the physiology of urban birds to their natural counterparts, very few have been performed on mammals (Stillman and Goss-Custard 2002, Møller 2009, Evans *et al.*, 2010, Bonier 2012, Powell *et al.*, 2013). Results from this study will thus constitute a framework that can be applied to the study of other small urban mammals.

Methods

Study site

Chipmunks were collected and sampled from two different habitats defined as urban and natural. Two urban areas, Huntsville and Sudbury, Canada were chosen for urban sites, and were paired with natural areas in Algonquin Provincial Park, and outside Sudbury respectively. A list of sites used can be found in Table 1.1. Urban habitats (located in and around cities) were chosen based on proximity to roads and walking trails, and their proximity to anthropogenic infrastructure (i.e. roads, fences, city structures), but where animals would come into contact with human activities. Natural habitats were selected in areas away from major human settlements and in forested areas where animals could escape human interaction and were not noticeably separated from outside populations by anthropogenic barriers.

Trapping

Trapping took place between May and August in 2012 and 2013. Trapping occurred during alternating weeks between urban and natural sites when possible. Chipmunks were trapped using Longworth live traps baited with sunflower seeds soaked in water overnight. Traps were set late morning and checked approximately 2 hours after. Approximately 60 traps were used per trapping session. Juveniles were not used in the analysis as they show consistently different reactions to stressful stimuli compared with adults (Boissy 1995, Bonier 2012). Captured individuals were transferred to a handling bag and were sexed and assessed for reproductive condition (reproductive or non-reproductive). Animals were classified as juvenile if their weight was below 80 g at initial capture and they were non-reproductive (Patterson and Schulte-Hostedde 2011). Individuals were marked with one or two metal ear tags to identify them with unique numerical codes. Juveniles and

other non-target species were immediately released. Callipers were used to measure skull length (length of back of skull to tip of nose +/- 1mm), skull width (length of skull between each eye +/- 1mm), and hind foot length (length of heel to the tip of the longest toe +/- 1mm). Blood for leukocyte analysis was collected by clipping a left hind toenail to the quick from each animal, and then placing a drop of blood on a slide and smeared using a second a slide in the field. The slides were labeled with the corresponding tag number form the sampled animal. The dried smears were stored at room temperature in a slide holder until further analysis.

Behaviour

Handling bag test

The handling bag test was conducted immediately once an individual was removed from the trap, tagged, and identified as an adult. The handling bag test provided a measure of an animal's docility; the less time spent struggling/moving in the bag, the more docile the animal (Martin and Réale 2008 b). The handling bag was held at arm's length for 1 minute (measured with a stopwatch) and the amount of time (in seconds) the animal spent moving in the bag was recorded.

Open Field test

An open field test is an effective method to measure an animal's activity in response to stressful situations; in particular the distance traveled in a novel environment (Montiglio *et al.*, 2010). The open field test was conducted immediately following the handling bag test. The test was performed in an opaque plastic arena (76 cm × 42 cm at the bottom and 90 cm × 53 cm at the top; height = 42 cm) with a clear, acrylic lid and 10 equally spaced holes (5 cm diameter) cut into the

bottom. A grid of squares (10 cm × 10 cm) was taped to the underside of the Plexiglass floor (Semenova *et al.*, 2001). A hollow tube with cover was placed in the box to allow animals to enter and be removed from the box. Once the chipmunk entered the enclosure its behaviour was recorded via video camera (HD, 1920 x1080) for 3 minutes (Montiglio *et al.*, 2010). All videos were scored by a single observer who remained silent and out of sight for the duration of the test. The videos were scored on time spent grooming, time spent head scanning, time spent running around the field box, time spend investigating each hole in the trap, and time spent inside the hollow tube. Additional scores were taken based on the number of times that chipmunks crossed lines within the grid as a measure of activity, and the number of head dips made by each animals as a measure of exploratory behaviour (Patterson and Schulte-Hostedde 2011). Chipmunks show degree of habituation to repeated open field trails (Martin and Reale 2008); therefore, only initial video trails were used in the analysis.

Stress

Hair/fecal Cortisol

Hair samples were collected by shaving a small 1 cm by 1 cm patch of hair from the right hind leg of each animal with a battery powered razor (ConAir Beard and Moustache Trimmer model GMT100RQCS, Stamford, CT) during the initial capture. Hair accumulates glucocorticoids over a period since the animal's last moult, and taking hair samples is a non-invasive method to measure the hormone levels without the risk of spiking glucocorticoid levels in plasma due to the stress caused by handling (Macbeth *et al.*, 2010, Ashley *et al.*, 2011). Hair is a relatively stable medium noted to maintain levels of blood based hormones for periods of weeks to months (Macbeth *et al.*, 2010, Ashley *et al.*, 2011). Hair samples were kept in 1.5 mL eppendorf tubes at room temperature.

Faecal samples were collected from a pillow case placed underneath the animal during each capture whenever possible. Faecal samples were collected into one 1.5ml eppendorf tube and placed in a solution of 80% methanol (with a concentration of approximately 0.100g faeces to 1mL methanol) and kept refrigerated. The faecal samples were weighed ± 0.001g. Methanol volumes were measured with a 10 mL graduated cylinder in 2012 and using a 1000 µl pipette (Fisher Scientific ISO/IEC 17025) in 2013. Fecal samples represent an accumulation of cortisol over a period of the animal's digestion, typically over a period of hours or days, though less when the animal is exposed to an immediate stressor such as handling (Ashley *et al.,* 2011).

All experimental procedures were in accordance with guidelines from the Canadian Council on Animal Care and were approved by the Animal Care Committee at Laurentian University (AUP 2012-01-04).

Lab Analysis – Hormone Extraction

All hair and faecal samples were sent to the endocrinology lab at the Toronto Zoo to undergo hormone extraction and enzyme immunoassay (EIA) to determine cortisol concentrations.

Hair samples

The hair samples were cut into 5mm pieces and placed into a 7mL glass scintillation vial and weighed using a four decimal place Mettler AB54-S chameleon balance. Mettler Toledo Rainin pipettes, both 1000µL and 200µL, were used to measure and transfer any liquids. Hair samples were vortexed for 10 seconds in a 100% methanol wash, which was pipetted off immediately after. The methanol wash was performed to remove any contaminants from the surface of the hair. Then, 80% methanol in water was added to extract cortisol from chipmunk hair at a ratio of 0.005g hair to 1mL methanol and samples were vortexed for 5-10 s. Cortisol was extracted from the hair into the

methanol for 24 hrs. at 100 r.p.m. on a Barstead Lab-Line Multi-Purpose Rotator. Each sample was centrifuged for 10 minutes at 35000 r.p.m. to concentrate hair at the bottom of the glass scintillation vials. Methanol extract (the supernatant) was moved to new 7mL glass scintillation vials, to be dried down in a fume hood. The dried extracts were stored at -20^{o}C until analysis.

Faeces samples

Immediately prior to extraction, the faecal pellets within each Eppendorf tube were broken up with a clean spatula and mixed with the methanol. The samples were vortexed briefly and hormone extraction was done overnight at 100 r.p.m. on a Barstead Lab-Line Multi-Purpose Rotator. Each sample was centrifuged for 10 m at 35000 r.p.m. to concentrate faeces at the bottom of the glass scintillation tubes. Methanol extract (the supernatant) was transferred into new 7mL glass scintillation vials. The extracts were stored at -20^{o}C until analysis.

Cortisol Enzyme Immunoassay

Samples were removed from the freezer and brought to room temperature on the lab bench prior to analysis. Dried hair extracts were reconstituted by adding assay buffer and sonicating for 20 sec followed by vortexing for 10 sec. The samples were centrifuged for one minute at 35000r.p.m., immediately before the EIA was run.

Hair cortisol and faecal cortisol metabolites were quantified using a method modified from Munro and Lasley (1988). Cortisol antiserum (R4972; C. Munro, University of California, Davis, CA, USA) was diluted in coating buffer (50mM bicarbonate buffer, pH 9.6) at 1:12, 000. The cross-reactivities of the cortisol antiserum were: cortisol, 100%: prednisolone, 9.9%: prednisone, 6.3%: cortisone, 5%: corticosterone, 0.7%: 21-deoxycortisone, 0.5%: deoxycorticosterone, 0.3%: other, <0.3%. Cortisol-horseradish peroxidase conjugate (C. Munro, University of California, Davis, CA, USA), was diluted in assay buffer (0.1mM sodium phosphate buffer, pH 7.0, containing 9g of NaCL

and 1g of bovine serum albumin per litre) at 1:60, 000. The standard used was cortisol (Steraloids Inc., Newport, RI, USA: cat # Sigma H-0135: 0.078 – 20 ng/mL = 78 – 20 000 pg/mL). Controls consisted of laboratory stocks of pooled faecal extracts obtained from female spotted necked otters (*Hydrictis maculicollis*) and run at 30% and 70% binding.

Microtitre plates (Nunc Maxisop, VWR, Mississauga, ON, Canada) were coated with 50µL of cortisol antibody diluted in coating buffer and incubated overnight at 4ºC. Unbound antiserum was washed three times from coated plates with 0.02% Tween 20 solution using a microplate washer (Bio-Tek Instruments, Winooski, VT, USA). Following the wash, 50µL of hair or faecal samples, standards, and controls diluted in assay buffer were added to wells in duplicate, followed by 50µL of cortisol-horseradish peroxidase conjugate diluted in assay buffer. Plates were incubated for two hrs at room temperature. Following incubation, the plates were washed three times and 100µL of substrate solution (50mM citrate, 1.6mM hydrogen peroxide, and 0.4mM 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, pH 4.0) was added. The intensity or absorbance of the yellow colour in each well on the microtitre was measured at 405nm using a spectrophotometer (MRX microplate reader, Dynex Technologies, Chantilly, VA, USA) 30-45 minutes after the substrate was added. The more yellow the well or the higher the absorbance, the lower the cortisol concentration. Cortisol levels are presented as ng of cortisol g^{-1} of hair or wet faeces.

Leukocyte analysis

Blood smears were sent to the Animal Health Care lab at the University of Guelph to be assessed for leukocyte type and abundance. The slides were stained using a hematek stainer with Modified Wright's stain. For a total leukocyte count estimate, the slides were analyzed using either a 40X or 100X objective. For the 40X, the mean number of leukocytes in 10 fields was multiplied by

1.5, and for the 100 X, the mean was multiplied by 8, to give the total white blood cell (WBC) count expressed as the approximate number of cells X $10^{9\ -1}$L. The leukocyte differential was determined by moving the field of view in a systemic pattern (zig zag) back and forth across the monolayer of the smear; individual leukocytes were counted until a total of 100 was reached (Davis *et al.*, 2008). The percentage of each leukocyte identified was then multiplied by the total WBC count to determine the absolute numbers for each leukocyte type.

Statistical Analysis

All statistical analysis were performed in R, using packages base (R Core Team 2014), nlme (Pinheiro et. al 2013), and candisc (Friendly and Fox 2013)

Open field test/ Handling bag trial

Video sampling was conducted on the initial open field trial for each animal, to assess an animal's response to being placed in a novel environment. I first performed a MANOVA using 9 of the 10 behavioural traits observed on the videos. Latency was removed from the analysis to correct for lack of independence between variables measured over a finite period of time. I then performed a linear discriminant analysis (lda) using the MANOVA results. I observed the lda loadings to determine the effect of each individual behavioral variable on the results. I finally used the results to conduct a linear mixed effect analysis with the results of the linear discriminant analysis as the dependant, and sex, reproductive condition (reproductive or non-reproductive), year of study, and habitat type (urban/natural) as the independent variables, and site as a random effect, to determine any within-site effects on the results. I performed the same linear mixed effect analysis on the handling bag trial times for each animal's initial capture, using them as the dependant variable.

Cortisol Concentrations

I $\log_{10}$ transformed the data to ensure normality and conducted a linear mixed effect analysis using cortisol concentrations as the dependent variable, and sex, reproductive condition (reproductive or non-reproductive), year of study, and habitat type (urban/natural) as the independent variables, and site as a random effect, to determine any within-site effects on the results. I conducted this test separately for both fecal and hair cortisol samples.

Leukocyte Counts/ratios

Under stressful circumstances, concentration of neutrophils in mammalian blood typically increases, while lymphocyte concentration decreases (Davis *et al.,* 2008). I therefore used a neutrophil/lymphocyte ratio, determined by dividing neutrophil percentages by lymphocyte percentages (Al-Murrani *et al.,* 2007). For the analysis of leukocyte ratios, I conducted a linear mixed effect using the neutrophil/lymphocyte ratios as the dependent variable, and sex, reproductive condition, year of study, treatment (urban/natural), and capture site as the independent variables, and site as a random effect. Similarly, I conducted a second linear mixed effect analysis with the leukocyte counts as the dependant variable, and sex, reproductive condition (reproductive or non-reproductive), year of study, habitat type (urban/natural), and habitat/sex interaction as the independent variables, and site as a random effect.

Results

A total of 140 individuals were sampled. Seventy-nine individuals were sampled in between May and August of 2012 (30 urban and 49 natural), and 51 individuals were sampled between May and August of 2013 (30 urban and 31 natural). Of those, 80 were collected from natural sites and 60 were sampled from urban sites. Fifty-one of the samples from natural sites were female (31 lactating/20 non reproductive), and 29 were male (14 scrotal and 15 non-reproductive). Thirty eight of the urban samples were female (15 lactating and 23 non-reproductive) and 22 were male (11 scrotal/11 non-reproductive). Not all individuals had all variables measured.

Handling Bag

A total of 126 initial handling bag trials were analysed. Habitat type (urban/natural) did not have a significant effect on the handling bag trials times ($t=-0.90$, $df=107$, $p=0.37$) (Table 1.2). However, trial times were significantly different between years ($t=8.98$, $df=107$, $p<0.001$) (Table 1.2).

Open Field Test

A total of 124 videos were assessed for the analysis. Behavioural variables analyzed are listed in table 1.3. Loadings of significant interest were locomotion and rearing (Table 1.4). Behavioural variables that contributed most to the results were movement (-0.41), which was displayed greater in natural habitats, and rearing (0.36), which was displayed greater in urban habitats (Table 1.4). Behavioural variables displayed in the open field test varied significantly between habitats (t-value= 5.20, DF=106, $p<0.001$) (Table 1.5).

Hair/Fecal cortisol

I collected hair from a total of 140 individuals. Habitat did not significantly influence hair cortisol concentrations (t=-0.49, p=0.63, df=121) (Table 1.6). There was a significant interaction effect between sex and habitat type (t=2.49, p=0.01, df=121) (Table 1.6). Year also had a significant influence on hair cortisol (t=0.91, p=0.03, df=121) with concentrations being higher during the second year of study (Table 1.6). Fecal samples were collected from 100 individuals. Fecal cortisol levels showed a trend of being higher in animals from natural habitats compared to urban ones (t=-1.71, p=0.09, df=78) (Table 1.7). Cortisol levels were significantly higher in reproductive individuals as compared to non-reproductive ones (t=2.86, p=0.01, df=78) (Table 1.7).

Leukocyte counts

A total of 98 samples were taken from individual chipmunks for leukocyte ratio analysis. Habitat had no influence on neutrophil/lymphocyte ratios (t=-1.21, p=0.23, df=79) (Table 1.8). A total of 87 individual samples were used in total leukocyte counts. No significant effect of habitat was detected on leukocyte totals (t=1.74, p=0.09, df=72), though a trend was present with total leukocyte counts being higher in urban animals (Table 1.9). Leukocyte counts were significantly higher in non-reproductive individuals (t=-2.15, p=0.03, df=72) (Table 1.9).

Discussion

Behaviour

No significant differences were reported in handling bag trial times between chipmunks from urban or natural habitats. However, initial reactions to an open field showed a significant increase of activity in natural individuals, and increase in exploratory behavior in urban individuals. Many similar studies show significant differences in boldness between urban and natural populations (Evans *et al.*, 2010), which lead me to predict that I would find similar results between these two study habitats. Locomotion in open field studies is often associated with high instances of activity behavioural traits (Martin and Reale 2008). My results are contradictory to my initial prediction that exploratory or activity behaviour would be higher in urban animals. In contrast, rearing was noted to be higher in urban sites. Rearing is typically associated with exploratory behaviour (Martin and Reale 2008), which is consistent with my predictions of exploratory behaviour being advantageous to urban animals. More exploratory animals make trades offs in finding new resources and potential mates at the cost of increased predation risk; however, the same can also be said for animals that show a high degree of activity (Martin and Reale 2008). Rearing can also be considered a reaction to habituation to human presence, as the animal is exploring its surroundings without viewing humans (the researcher) as a potential predator. Studies have shown urban chipmunks to react normally to humans who perform "normal behaviours", such as walking by and otherwise ignoring the chipmunk (Bateman and Flemming 2014).

Another possibility for my behavioural results is that insufficient differences exist between the urban and natural sites used for the experiment. Predators are present in both types of sites; domestic cats and dogs are noted predators of small urban mammals, whereas natural animals are preyed upon by a variety of wild predators. It is also possible that urban conditions were similar to those of natural conditions, in terms of habitat type and cover (Ditchoff *et al.*, 2006). Sudbury and

Huntsville are medium sized cities, relatively spread out and surrounded by large expanses of forest, which could lead to interactions between individuals from each habitat type and individuals with different behavioural traits to easily disperse between habitat types. Another explanation is that personality can influence the likelihood that individuals investigate and enter the traps used for testing. Boldness can positively influence capture (Dingemanse and Réale 2005, Patterson and Schulte-Hostedde 2011, Biro 2013). This may have created a bias in the animals that I captured and used in the study, which may explain the lack of differences in expressed personality traits between treatments, particularly during the handling bag test.

Stress-Cortisol Concentrations

In contrast to my original prediction, habitat had no significant effect on hair cortisol concentrations, and cortisol concentrations from fecal samples showed a trend of being greater in natural habitats than in urban centers. Several studies have shown increased cortisol levels in urban animals over natural conspecifics (Bonier *et al.*, 2007, Fokidis *et al.*, 2009); however, markedly different results do occur in the literature (Powell *et al.*, 2013). Urban animals that have access to an abundance of food resources are less likely to be stressed compared to natural conspecifics, especially in areas that are subject to seasonal food availability (Fokidis *et al.*, 2009, Bonier 2012). Similarly, birds fed with a diet high in proteins show an overall greater stress response than those fed with lower protein diets (Fokidis *et al.*, 2009). While no overall significant effect of habitat or sex were observed to influence hair cortisol concentrations, an interaction effect of habitat and sex was observed on cortisol levels. It is possible that my results were influenced by greater numbers of females trapped from non-urban habitats. Observations of my data show slightly higher cortisol concentrations in males samples from urban habitats. While many species show increased glucocorticoid levels as a result of anthropogenic stressors, others have adapted to down-regulate their stress response, limiting the negative effects of long terms stress exposure (Partecke *et al.*,

2006). Desensitization to a stressor can occur in instances where the organism is repeatedly exposed to the stressor (Fokidis *et al.,* 2009). This has been shown by a down-regulation of HPA activity to reduce glucocorticoid production in urban birds exposed to frequent stressors (Fokidis *et al.,* 2009). Similarly, mammalian neonates subjected to stress have shown an overall reduced stress response as a result and have subsequently shown reduced cortisol production into adulthood (Partecke *et al.,* 2006). Because of this it is possible that animals in natural habitats, in more stressful conditions, have adapted to long term stress by reducing their cortisol concentrations to a level similar to that of less stressed urban animals.

Another possible explanation for the lack of long term stress differences between habitats is the presence of similar conditions between both urban and natural sites that may cause long term stress. One such similarity might be predation risk. Humans are seen as a potential predator for many animal species (Marzluff *et al.,* 2001, Frid and Dill 2002, Stillman and Goss-Custard 2002, Møller 2009, Powell *et al.,* 2013); this combined with predation risk from domestic pets may contribute to similar levels of predation risk between the two types of sites.

Stress-leukocyte concentrations

The results of my leukocyte analysis did not show a significant effect of habitat on leukocyte counts from urban and natural sites. The results I observed show a lack of significant differences in the ratios of lymphocytes and neutrophils. Similarly, a lack of significant difference was detected in leukocyte totals between the two habitat types. An increase of neutrophil concentration and decrease of lymphocytes is typically associated with stress in mammalian leukocytes, as well as an overall increase in leukocyte totals (Nunn *et al.,* 2000, Davis *et al.,* 2008). Therefore, I would expect to see a significant increase in the neutrophil/lymphocyte ratio as well as in leukocyte totals in natural

chipmunks to correlate with my findings of increased cortisol production. However, as long term cortisol concentrations measured from hair did not differ between habitat types, it is possible that these leukocyte ratios and totals were not influenced by short term stress differences and thus did not appear significantly different between the two habitats. Leukocyte totals were significantly higher in non-reproductive individuals; however, the neutrophil/lymphocyte ratio was lower in non-reproductive individuals as compared to reproductive individuals, suggesting the increase is not a result of greater stress.

Future directions

The principal criteria I used for determining whether an animal was urban or natural was based on location and available territory. Few studies have looked at multiple species across several different cities (Bonier 2012). A larger sample size using more cities would be ideal; broad examinations of multiple cities performed by different studies showed significant differences in behaviours performed by urban birds compared with natural conspecifics (Blumstein *et al.,* 2003). Similarly, this data set represents only a two year snap shot of each sampled population; ideally a study over multiple years would show if these trends continued. Annual differences were detected in stress hormone levels; a study over multiple years would better illustrate the general trends in each of the sampled populations. Diet can significantly influence stress levels in urban centers; animals with a wide range of dietary options are less likely to show signs of stress (Fokidis *et al.,* 2009). Examining whether chipmunks prefer more nutrient rich food in urban centers as compared to more natural sites by examining chipmunk caches or the stomach contents of deceased individuals could show similar trends to other variables I assessed in my study such as cortisol concentrations. Finally, although the cities I chose could certainly be considered as urban habitats, perhaps it would be interesting to repeat the exercise in a larger, more intensely urban habitat. Huntsville and Sudbury

are small to medium cities in central Ontario and are bordered by large expanses of forest. Therefore, it is possible that significant gene flow from natural areas could mask advantageous urban traits such as boldness in behavioural expression. Hence, it could be worth to repeat the experiment in a larger metropolitan area such as downtown Toronto, and see whether this would yield differing results.

Conclusions

In conclusion, my results show some significant difference in behavioural patterns of urban chipmunks as compared with their natural counterparts; however, stress hormone levels remained similar between individuals from different habitats. Because behavioural traits of exploration and activity are often associated in behavioural studies, it is possible that habituation to human presence may explain the differences in activity and exploration I found between urban and natural habitats. While a trend of higher concentrations of cortisol existed from fecal samples taken from natural populations, no differences were detected in cortisol levels from hair samples. This suggests that natural animals experience some greater degree of stress, though stressors between habitats are similar, or that animals cross between habitats. Finally, leukocyte ratios and abundances did not differ between habitats, which correlates with my findings on long term cortisol production as observed from hair concentrations. Not all species respond similarly to urbanization (Fokidis *et al.*, 2009, Bonier 2012); therefore, trends evident in other species can be used to predict how others will respond, and it is important to assess individual species effects.

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Tables and Figures

Table 1.1 Coordinates of sites used to sample eastern chipmunks (*Tamias striatus*) sampled from Algonquin Provincial Park, Sudbury, and Huntsville Ontario

Site Name	Coordinates	Habitat type
Algonquin park one	45°32'N, 78°36'W	Natural
Algonquin park two	N 45° 32' 40.9065 , W 78° 41' 45.64	Natural
Algonquin park three	N 45° 33' 54.6862 , W 78° 33' 59.6101	Natural
Highway 144	N 46° 36' 30.2195, W 81° 24' 32.0229	Natural
Highway 17	N 46° 25' 27.0767, W 81° 9' 37.7079	Natural
Lake Laurentian conservation area	N 46° 27' 19.6303, W 80° 56' 51.1412	Natural
Huntsville	N 45° 20' 25.4029, W 79° 14' 12.1305	Urban
Huntsville	N 45° 19' 45.5451, W 79° 13' 19.1193	Urban
Huntsville	N 45° 19' 27.7858, W 79° 12' 31.4769	Urban
Laurentian campus	N 46° 28' 5.3785, W 80° 58' 19.4448	Urban
Sudbury 1	N 46° 28' 47.8514, W 81° 0' 25.0784	Urban
Sudbury 2	N 46° 26' 39.7066, W 81° 3' 20.7202	Urban

Sudbury 3	N 46° 28' 2.1214 W 80° 57' 28.7511	Urban
Sudbury 4	N 46° 26' 59.1406, W 80° 59' 16.4343	Urban

Table 1.2. Linear mixed effect analysis using model of behavioural variables from handling bag trial times results from eastern chipmunks (*Tamias striatus*) (N=126) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	df	p-value
Intercept	-23.90	6.98	-3.42	107	**<0.001**
Habitat	-4.66	5.19	-0.90	107	0.37
Reproductive condition	-3.71	3.21	-1.15	107	0.25
Sex	-0.29	3.38	-0.08	107	0.93
Year	35.83	3.99	8.98	107	**<0.001**

Table 1.3. Behavioural responses observed in eastern chipmunks (*Tamias striatus*) in an open field box trial from samples taken in Algonquin Provincial Park, Sudbury, and Huntsville Ontario.

Variable observed	Description
Locomotion	Time spent walking/running within the open field box
Head scanning	Time spent scanning head back and forth while remaining otherwise still
Grooming	Time spent grooming or scratching
Jumping/climbing	Time spent jumps to top of the container or remains hanging from top of container or entrance
Head dipping	Time spent with entire head lowered in holes within open field box
Rearing	Time spent rearing up on its hind legs viewing its surroundings
Still	Time spent completely motionless visible in the open field box
Latency	Time spent out of sight in the entrance
# Head dips	Number of times head dipped into a hole
#Line crossings	Number of times animal crosses a line on the floor of the open field box with its entire body

Table 1.4: A summary of loadings from a linear discriminant analysis (lda) of expressed behavioural traits observed during open field trials of eastern chipmunks (*Tamias striatus*) (N=124) sampled from Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Components retained for further analysis (Kaiser-Guttman criterion) are in bold.

Behaviour	Loadings
Locomotion	**-0.41**
Head scanning	0.07
Grooming	-0.15
Jumping/climbing	-0.26
Head dipping	-0.16
Rearing	**0.36**
Still	-0.07
Number of head dips	- 0.13
Number of line crossings	0.14

Table 1.5. Linear mixed effect analysis using scores from candisc analyses in R, on behavioural variables observed from an open field behavioural trials collected from eastern chipmunks (*Tamias striatus*) (N=124) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	Df	p-value
Intercept	-0.35	0.30	-1.16	106	0.25
Habitat	0.94	0.18	5.20	106	**<0.001**
Reproductive condition	0.10	0.18	0.54	106	0.59
Sex	-0.12	0.18	-0.65	106	0.52
Year	-0.08	0.18	-0.43	106	0.67

Table 1.6. Linear mixed effect analysis on cortisol levels in hair collected from eastern chipmunks (*Tamias striatus*) (N=140) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	Df	p-value
Intercept	1.65	0.19	8.56	121	**<0.001**
Habitat	-0.05	0.12	-0.49	121	0.63
Reproductive condition	0.01	0.06	0.23	121	0.82
Sex	0.01	0.09	0.12	121	0.90
Year	0.1	0.11	0.91	121	**0.03**
Habitat*Sex	0.31	0.12	2.49	121	**0.01**

Table 1.7. Linear mixed effect analysis on cortisol levels in fecal samples collected from eastern chipmunks (*Tamias striatus*) (N=134) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	df	p-value
Intercept	2.37	0.15	16.29	78	**0.00**
Habitat	-0.20	0.12	-1.71	78	0.09
Reproductive condition	0.18	0.06	2.86	78	**0.01**
Sex	-0.05	0.06	0.08	78	0.94
Year	-0.09	0.08	-1.16	78	0.25

Table 1.8. Linear mixed effect analysis on neutrophil/lymphocyte ratios in blood collected from eastern chipmunks (*Tamias striatus*) (N=98) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario.

	Estimate	Std.Error	Df	t-value	p-value
Intercept	6.81	4.29	79	1.59	0.12
Habitat	-3.94	3.26	79	-1.21	0.23
Reproductive condition	-2.05	2.00	79	-1.03	0.31
Sex	-4.22	3.04	79	-1.39	0.17
Year	-0.54	2.34	79	-0.23	0.12

Table 1.9. Linear mixed effect analysis on leukocyte totals in blood collected from eastern chipmunks (*Tamias striatus*) (N=87) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	df	t-value	p-value
Intercept	0.50	0.15	72	4.67	**0.00**
Habitat	0.10	0.06	72	1.74	0.09
Reproductive condition	-0.12	0.06	72	-2.15	**0.03**
Sex	-0.01	0.06	72	-0.24	0.81
Year	0.10	0.06	72	1.74	0.09

Chapter 2

The effect of long term urbanization on body condition and endoparasite burden in eastern chipmunks (*Tamias striatus*)

Abstract

Urban centers are generally comprised of isolated habitat fragments with wildlife populations at relatively high density. Animals that exist in urban habitats have access to greater food resources than their natural conspecifics, but generally these food sources provide poor nutritional quality and can lead to reductions in health and body condition. Furthermore, urban animals in high densities are subject to greater rates of disease and parasitism, which can have many long term fitness consequences. I tested the prediction that urban chipmunks would have an overall poor body condition when compared to their natural conspecifics. Furthermore, if a reduction in body condition is observed in urban chipmunks, a greater rate of parasitism should also be observed, reflected in higher diversity and volume of fecal oocytes, and increased concentrations of leukocytes (eosinophils). I used Huntsville and Sudbury, Ontario for my urban sites, and Algonquin Provincial Park and areas outside Sudbury for my natural sites. I live trapped chipmunks, and took measurements of skull length, width, and hind foot length to determine body condition. I collected fecal samples for oocyte assessments and blood smears were used for analysis of leukocyte counts. Contrary to my prediction, results showed that urban individuals had significantly greater body condition that their natural conspecifics. However, no significant differences in parasitism rates in terms of type and presence of oocytes were observed. These results suggest that food quality in the urban sites chosen was superior to that consumed by natural individuals, but that interactions exist between the urban and natural populations in my study, which would allow transfer of parasites between habitats and explain the similar oocyte trends I observed.

Introduction

Urban centers fragment habitats, destroying or modifying the original landscape and creating novel selective pressures that can influence rapid evolutionary change in many animal species (Stockwell *et al.,* 2003, Adams 2005, Sih *et al.,* 2011, Harris *et al.,* 2013). Habitat fragmentation increases edge effects and predation rates, and reduces species abundance (Nupp and Swihart 1998, Fernandez-Juricic 2000, Stockwell *et al.,* 2003, Adams 2005, McGregor *et al.,* 2008, Harris *et al.,* 2013). Urban animals are often isolated from other populations by natural and anthropogenic barriers, which have the potential to limit gene flow and create genetic drift (McGregor *et al.,* 2008). These fragments create pockets of habitat where resident species can exist in highly dense populations (Nupp and Swihart 1998, Stockwell *et al.,* 2003, Adams 2005, Shochat *et al.,* 2006, Liker *et al,.* 2008, Møller 2009, Harris *et al.,* 2013). These changes in population structures from fragmentation over many subsequent generations of animals can have evolutionary consequences and affect phenotypic variation.

Effect of urbanization on body condition

Body condition, defined as the energetic state of an animal (Schulte-Hostedde *et al.,* 2005) is associated with many behavioural, physiological, and life history traits in animal populations. An animal in good body condition is expected to have more energy reserves and thus have a higher rate of survival than an animal with a poor body condition (Schulte-Hostedde *et al.,* 2005). In natural settings, a good body condition is particularly adaptive, especially when food resources are low (Schulte-Hostedde *et al.,* 2005, Liker *et al.,* 2008). However, in urban centers, animals with a poor body condition are able to survive on a food supply that is generally predictable and available year round from anthropogenic sources (Shochat 2004, Liker *et al.,* 2008). The credit card hypothesis predicts that animals in urban centers likely store fewer resources than natural conspecifics, living on

the "credit" of readily available food from anthropogenic sources (Shochat 2004, Liker *et al.,* 2008, Powell *et al.,* 2013). For example, passerine birds in urban centers have been shown to have up to 5% less body mass and smaller skeletons compared to their rural counterparts (Liker *et al.,* 2008).

Animals in urban populations are also subject to high levels of toxic chemicals and metals from anthropogenic food and waste sources (Shochat 2004, Liker *et al.,* 2008, Sih *et al.,* 2011). Many of these food sources have poor quality and are insufficient for growth and development for juvenile animals (Shochat 2004, Mennechez and Clergeau 2006). Exposure to high levels of pollutants and metals has been found in urban birds, and has been associated with lower body condition when compared to animals not exposed to the same pollutants and metals (Janssens *et al.,* 2003). For instance, juvenile gulls that are fed anthropogenic food waste are noted to have lower fledging rates than those fed fish caught at sea (Shochat 2004).

Effect of urbanization on parasite loads

High population densities facilitate parasite and disease transmission, which can influence an organism's health and body condition by diverting resources from growth and reproduction towards immune function (Neuhaus 2003, Sih *et al.,* 2004, Grear *et al.,* 2009, Hillegass *et al.,* 2010, Gooderham and Schulte-Hostedde 2011). Furthermore, urbanization changes the local ecosystem by limiting the number of species and selecting for species that can adapt to the urban context. Changes in species composition and number of competitors can alter food webs and interactions, and subsequently alter pathogens and immune defences present in the population (Harris *et al.,* 2013).

Parasites increase their own survival with a variety of immunosuppressant strategies within their host species (Barnard *et al.,* 1998) and these secondary effects of parasitism can impede host growth and reproduction either directly or indirectly via resource allocation (Sheldon and Verhulst 1996, Figuerola *et al.,* 1999, Gooderham and Schulte-Hostedde 2011). Poor nutrition, as experienced in urban populations is associated with a decrease in immune function and thus an increased

susceptibility to parasitism (Sheldon and Verhulst 1996, Figuerola *et al.,* 1999). Parasitism can also decrease attractiveness to a potential mate (Neuhaus 2003, Hillegass *et al.,* 2010, Gooderham and Schulte-Hostedde 2011). For example, reproductive success was notably higher in female cape ground squirrels treated for ectoparasites and endoparasites (Neuhaus 2003, Hillegass *et al.,* 2010).

Increases in parasite load are also reflected in leukocyte abundances and ratios. Five types of leukocytes are found in most vertebrate taxa: lymphocytes, monocytes, neutrophils, basophils, and eosinophils, and each leukocyte type has a specific function (Davis *et al.,* 2008). In mammals it has been observed that concentrations of eosinophils increase in response to high rates of disease and parasite infection (Nunn *et al.,* 2000, Dhabhar 2002, Davis *et al.,* 2008).

Study species

The eastern chipmunk, *Tamias striatus,* is a small rodent species native to much of eastern and central Canada and the United States. They have wide home ranges that show overlap between males, females, and juveniles (Blair 1943). They have been used in a wide variety of studies to assess behavioural changes to environmental stimuli (Patterson and Schulte-Hostedde 2011, Martin *et al.,* 2011). Chipmunks have displayed a high degree of repeatability and recapture success, which make them an ideal model for this study (Yerger 1955, Martin *et al.,* 2011, Patterson and Schulte-Hostedde 2011).

The purpose of this study was to determine the long terms effects of urbanization on body condition and parasitism rates in populations of *T. striatus*. While similar studies have been performed on urban birds compared to their natural counterparts, few have been performed on mammals (Stillman and Goss-Custard 2002, Møller 2009, Evans *et al.,* 2010, Bonier 2012, Powell *et al.,* 2013). Body condition was used as a measure to determine differences in energy reserves between urban and natural chipmunk populations. I predicted that urban chipmunks will have poorer

body condition compared to chipmunks in natural habitats, due to poor food quality and high population densities found in urban centers. Similarly, due to high densities in urban centers (Møller 2009), I predicted higher degrees of parasite infection in urban centers as compared to natural habitats. Furthermore, I also predicted a higher abundance of eosinophils from leukocyte count data in urban animals in relation to higher rates of parasitism in urban centers.

Methods

Study site

Chipmunks were collected and sampled from two different habitat types defined as urban and natural. Two urban areas, Huntsville and Sudbury, Canada were chosen for urban sites, and were paired with natural areas in Algonquin Provincial Park, and outside Sudbury respectively. A list of sites used can be found in Table 2.1. Urban habitats (located in and around cities) were chosen based on proximity to roads and walking trails, and their proximity to anthropogenic infrastructure (i.e. roads, fences, city structures), where animals would come into contact with human activities. Natural habitats were selected in areas away from major human settlements and in forested areas where animals could escape human interaction and were not noticeably separated from outside populations by anthropogenic barriers.

Trapping

Trapping took place between May and August in 2012 and 2013. Chipmunks were trapped using Longworth live traps baited with sunflower seeds soaked in water overnight. Traps were set late morning and checked approximately 2 hours after. Approximately 60 traps were used per

trapping session. Juveniles were not used in the analysis as they show consistently different reactions to stressful stimuli compared with adults (Boissy 1995, Bonier 2012). Captured individuals were transferred to a handling bag and were sexed and assessed for reproductive condition (scrotal, oestrus/lactating, or non-reproductive). Animals were classified as juvenile if their weight was below 80 g at initial capture and they were non-reproductive (Patterson and Schulte-Hostedde 2011). Individuals were marked with one or two metal ear tags to identify them with unique numerical codes. Juveniles and other non-target species were immediately released. Callipers were used to measure skull length (length of back of skull to tip of nose +/- 1mm), skull width (length of skull between each eye +/- 1mm), and hind foot length (length of heel to the tip of the longest toe +/- 1mm). Blood for leukocyte analysis was collected by clipping a left hind toenail to the quick from each animal, and then placing a drop of blood on a slide and smeared using a second a slide in the field. The slides were labeled with the corresponding tag number from the sampled animal. The dried smears were stored at room temperature in a slide holder until further analysis.

All experimental procedures were in accordance with guidelines from the Canadian Council on Animal Care and were approved by the Animal Care Committee at Laurentian University (AUP 2012-01-04).

Parasite load - Fecal egg counts

An estimate of internal parasite load was made using the number of eggs per gram of wet feces, using a modified McMaster technique (Patterson and Schulte-Hostedde 2011). Fecal samples were blended using a solution of zinc sulphate (Ricca chemical company) at a 1:10 dilution. The mixture was then strained through a sieve to separate matter, and then poured into a 100ml tube and shaken thoroughly. Each tube was left for 10 minutes to allow the eggs to float to the top of the mixture. One millilitre (1ml) of solution was extracted and placed into two chambers of a McMaster

slide, and examined under a microscope (10x magnification; Olympus BX41, Tokyo, Japan). The eggs were counted within two 1cm grids. The number of eggs per gram of feces (epg) was calculated by multiplying the sum of the number of eggs observed within each chamber by 100. Egg counts were averaged between the two grids and then for multiple captures of the same individual over the course of the study. Eggs were identified using several online archives and identification guides; Diagnosing medical parasites (http://www.afpmb.org/sites/default/files/whatsnew/2009/Diagnosing_Medical_Parasites.pdf), The RVC/FAO guide to veterinary diagnostic parasitology (http://www.rvc.ac.uk/review/Parasitology/EggID/EggID.htm), Laboratory animal medicine and science series 2 (http://ehs.uc.edu/lams/data/pdfs/9045.pdf), A-Z guide to parasitology (http://www.southampton.ac.uk/~ceb/Diagnosis/Vol5.htm), and clinical parasitology images and description (http://instruction.cvhs.okstate.edu/jcfox/htdocs/clinpara/Index.htm).

Parasitism impacts- Leukocyte analysis

Blood smears were sent to the Animal Health Care lab at the University of Guelph to be assessed for leukocyte type and abundance. The slides were stained using a hematek stainer with Modified Wright's stain. For a total leukocyte count estimate, the slides were analyzed using either a 40X or 100X objective. For the 40X, the mean number of leukocytes in 10 fields was multiplied by 1.5, and for the 100 X, the mean was multiplied by 8, to give the total white blood cell (WBC) count expressed as the approximate number of cells X $10^{9\ -1}$L. The leukocyte differential was determined by moving the field of view in a systemic pattern (zig zag) back and forth across the monolayer of the smear; individual leukocytes were counted until a total of 100 was reached (Davis *et al.*, 2008). The percentage of each leukocyte identified was then multiplied by the total WBC count to determine the absolute numbers for each leukocyte type.

Statistical analysis

All statistical analysis were performed in R, using packages base (R Core Team 2014), nlme (Pinheiro et. al 2013), and ggplots2 (Wickham.2009).

Body condition

Three measured traits were used to determine body size; hind foot length, skull length, and skull width (Schulte-Hostedde *et al.,* 2005). I used the average of each measurement for each individual. Each trait was then $\log_{10}$ transformed to maintain normality. I conducted a principal component analysis using the measured traits skull length, skull width and hind foot length to reduce variation. A linear regression was then run with mass as the dependent variable and the PC1 as the independent variable. The residuals were then determined and a linear mixed effect model was performed using the new calculated residuals as the dependant variable, and treatment (urban or natural), sampling year, reproductive condition (reproductive or non-reproductive) and sex as the independent variables, and site as a random effect.

Fecal egg counts

The oocytes observed were classified by type (based on differences in morphology), as oocytes could not be identified to species. The total number of oocyte types per individual (richness), the number of individuals infected with a particular oocyte (prevalence), as well as the total number of eggs per individual was calculated. A linear mixed effect model was conducted using habitat (urban or natural), sampling year, reproductive condition (reproductive or non-

reproductive) and sex as the independent variables, capture site as a random effect, and using richness as the dependant variable. A separate linear mixed effect model was conducted to determine the effect of the same independent variables on the total numbers of eggs observed. For prevalence, I scored each individual chipmunk with a 0 (uninfected) or 1 (infected) for each oocyte type identified (Hancke *et al.,* 2011). I then used a logistic regression model with prevalence as the dependant variable, and habitat (urban or natural), sampling year, reproductive condition (reproductive or non-reproductive) and sex as the independent variables.

Leukocyte Abundance

As eosinophil percentages typically increase in response to parasitism effects (Davis *et al.,* 2008), I used the percentage of eosinophils in leukocyte counts as a measure of parasitic infection. I performed a logistic regression using the proportion of eosinophils as the dependent variable, and sex, reproductive condition (reproductive or non-reproductive), year of study, and habitat (urban/natural) as the independent variables.

Results

A total of 140 individuals were sampled for this project. Seventy-nine individuals were trapped in between May and August of 2012 (30 urban and 49 natural), and 51 individuals were trapped between May and August of 2013 (30 urban and 31 natural). Of those, 80 were collected from natural sites and 60 were sampled from urban sites. Fifty one of the natural samples were female (31 lactating/20 non reproductive), and 29 were male (14 scrotal and 15 non-reproductive).

Thirty eight of the urban samples were female (15 lactating and 23 non-reproductive) and 22 were male (11 scrotal/11 non-reproductive).

Fecal Oocyte counts

Fecal samples from 84 individuals were used in this analysis. Seven separate types of eggs were identified using online references: cestode (unknown type), Entamoeba *sp,* nematode (*Ricularia sp.)* pinworm (*Syphacia sp*) as well as three unknown egg types. The linear model analysis indicated that habitat did not significantly influence oocytes totals (t=-0.1, p=0.93, df=78) (Table 2.2). The linear model analysis result showed that richness varied significantly between years of study (t= -2.91, p< 0.05, df = 78) (Table 2.3). On the other hand, habitat had no significant effect on richness (t=0.32, p=0.75, df=78) (Table 2.3). Similarly, prevalence did not vary significantly between habitat type (t=0.14, p=0.89) (Table 2.4); however, prevalence varied significantly between the 2012 and 2013 sampling years (t=-3.11, p<0.05) (Table 2.4).

Body condition

Body measurements were taken from a total of 133 individuals (Table 2.5). The principal component analysis on body measurements shows that PC1 explained 50% of the variance, and was positively correlated with hind foot length (0.46), skull length (0.29), and skull width (0.45). Thus, PC1 was used as an index of body size. The results of the linear model of PC1 on body mass were significant (t=3.77, p<0.001 (Table 2.6). Body condition was significantly higher in urban individuals (t=4.37, p<0.001, df = 117) (Figure 2.6), as well as reproductive individuals (t=6.61, p<0.001, df=117) (Table 2.6), and animals captured in the second year of study (t=3.95, p<0.001, df=117) (Table 2.6). The variables habitat and sex also interacted significantly (t=-1.94, p=0.05, df=117) (Table 2.6)

Leukocyte counts

A total of 98 blood smear samples were taken from chipmunks and used for the leukocyte analysis of eosinophil percentages. Overall, no effect of habitat was seen on eosinophil percentages ($t=1.52$, $p=0.13$, $df=97$) (Table 2.7).

Discussion

Body Condition

My findings on body condition differences did not support my initial hypothesis that body condition would be superior in natural animals compared to their urban conspecifics. Overall, a significant difference in body condition was noted between urban and natural individuals; however, urban individuals appeared to show a superior body condition, as opposed to my initial prediction. Studies on body condition between urban and natural individuals have generally shown mixed results. Liker *et al.* (2005) found a 5% decrease in body condition in urban house sparrows when compared to natural conspecifics. They predicted that a few individuals were urban exploiters and consumed far more than needed because food resources were readily available, leaving a minimum amount of food available for the majority of urban individuals (Shochat 2004, Liker *et al.,* 2008). Similarly, animals that engage in feeding and fat storage make trade-offs between reducing risk of starvation and reducing ability to escape predation (Cuthill *et al.,* 2000). As urban animals have access to regular dependable food resources, it would therefore be more beneficial for urban animals to consume only what is needed for survival since the risk of starvation is minimal.

One possible scenario to explain my results may be that minimal predation in urban centers (Møller 2009) would offset the costs of high body mass. Animals could consume more than necessary due to the abundant availability of food resources in urban centers and could store those additional resources as fat with a reduced risk of being caught by predators only encountered by conspecifics in natural habitats (Møller 2009); although predation risk from domesticated urban animals may still exist. Another possibility is that food quality in the urban centers used for this study was overall superior than food consumed by natural conspecifics. Generally, it is expected that although food abundance is higher in urban centers, food generally comes from anthropogenic sources and has poor nutritional value (Mennechez and Clergeau 2006). This is shown in poor juvenile body condition in many studies involving urban birds (Mennechez and Clergeau 2006). However; in studies where urban food quality was higher than in natural sites, body condition was scored as relatively higher within urban centers as compared to natural ones (Fokidis *et al.,* 2009). Further comparative analysis of chipmunk dietary preferences between the two habitat types may shed some light on this. A third possibility takes into account the assumption that food sources in urban centers are predictable and consistent. Starlings (*Sturnus vulgaris)* have been shown to store food and increase their mass and body condition score, likely due to anticipation of seasonal variation (Cuthill *et al.,* 2000). This is however unlikely for the chipmunk populations I studied as most of the trapping occurred in the summer months, where food predictability would be expected to be the greatest and most consistent. Finally, as chipmunks cache food for winter months (Yerger 1955), it is possible that due to greater food availability in under centers, urban chipmunks were able to cache more food than natural conspecifics. This would allow access to greater food resources in winter months which would lead to an overall superior body condition. Therefore it would be of interest to compare chipmunk cache stores from both urban and natural populations.

Parasite impacts

Parasitism generally reduces the resources available for the host organism to perform other vital functions via a number of direct and indirect effects (Pacejka *et al.,* 1998, Bouslama *et al.,* 2002). I predicted that due to high population densities and limited dispersal between populations, animals in urban areas would suffer greater rates of parasite infection than natural ones. Specifically, I predicted that both richness and total numbers of oocytes would be significantly higher in urban populations. My results did not support these predictions as there were no significant differences in parasitism rates between chipmunks from urban and natural habitats. Numerous host life history traits can influence parasite communities, including population size, habitat, diet, stress, host immune response, interactions between parasite species within a host, and dispersal (Pacejka *et al.,* 1998, Figeurola *et al.,* 1999, Poulin and Valtonen 2002). Another factor is that in urban centers, anthropogenic barriers such as roads are expected to limit interaction between urban populations and those from surrounding areas (McGregor *et al.,* 2008). However, the urban areas chosen for this study are bordered by large expanses of forest, and the urban areas themselves were relatively small. If some degree of interaction occurred between the studied urban and surrounding natural chipmunk populations, urban animals would be exposed to parasites found in natural populations and vice versa. Small mammals such as chipmunks prefer to avoid road surfaces, however, some individuals will cross roads, particularly to return when translocated, and traffic rates have not shown a significant effect on road crossing rates (McGregor *et al.,* 2008). Furthermore, many common endoparasite species have been detected in both urban and natural population of other small mammals such as the brown rat (*Rattus norvegicus*) (Hancke *et al.,* 2011), suggesting that

interactions could occur between urban and natural populations that could facilitate parasite transmission.

Animals have a finite pool of energy resources to draw from, and it is expected that highly parasitized animals would have to utilize resources from other functions in order to successfully trigger their immune defence strategies (Gooderham and Schulte-Hostdde 2010, Hancke *et al.,* 2011). Furthermore, because decreases in body condition are also typically associated with parasitic infection (Figeurola *et al.,* 1999, Hancke *et al.,* 2011, Bonier 2012); I expected to find a similar trend between parasitism rates and body condition scores. Animals have developed a wide array of defences to reduce the effects of parasitism, based on a ratio of parasitism impacts versus the cost to maintain such defences (Sheldon and Verhulst 1996, Schmid-Hemple 2003). One possibility is that urban animals have a significant greater spleen mass as compared to their natural conspecifics, as a means to help better cope with high endoparasite infection rates (Møller 2009). Such adaptations may explain how no significant differences in parasite loads were observed between two different habitat types when it is expected that one habitat type should have significantly higher rates of parasitism. Similarly, several studies report no noticeable effect of reduced parasite load on life history traits such as reproductive success (Pacejka *et al.,* 1998, Dowling *et al.,* 2001, Bouslama *et al.,* 2002). It has been hypothesized that in some cases the costs of higher rates of parasitism are offset by behavioural modifications such as avoidance of infected areas or individuals, increased food gathering, or providing plants or nutrients sources that stimulate immune function (Bouslama *et al.,* 2002). In some instances, poor body condition is independent of parasitism, particularly when body condition was previously poor from other factors such as lack of food or breeding season stressors (Figeurola *et al.,* 1999). Similarly, some parasites do not have immediate effects on their host species, such as the blood parasite *Plasmodium* in birds, but may have long term fitness consequences not detectable in body condition changes (Figeurola *et al.,* 1999). Therefore, it is

possible for two populations with similar parasite loads to display differences in body condition, particularly if one population has access to greater food resources and reduced predation, which may be the case in the animals sampled in these urban habitats.

Typically, concentrations of eosinophils increase in response to high parasitic infection (Davis *et al.*, 2008, Figeurola *et al.*, 1999). I expected that percentages of eosinophils would be greater in blood smears taken from urban individuals. However, analysis of the chipmunk leukocyte data did not show a difference between eosinophil concentrations between either habitat types. Parasitism rates as measured through oocyte prevalence, richness and oocyte totals were not significant between the two habitat types; therefore it can be expected that eosinophil concentrations would not differ significantly as well. Concentrations of eosinophils in lymphocyte counts can also be influenced by a number of factors such as a recent infection or age of the infected individuals (Figeurola *et al.*, 1999).

Future Directions

One way to answer some of the questions raised in this study would be to assess endoparasite infection by collecting deceased individuals or euthanizing individuals to sample intestinal contents of endoparasites. Assessment of adult parasites would provide a more accurate picture of each animals intestinal parasite load to determine if any differences existed between the two habitats. Another aspect of interest might be to determine the dietary preferences of urban individuals as compared to individuals in natural areas, perhaps by examining stomach contents of euthanized individuals or by locating and examining a chipmunk food cache. Higher food quality in urban centers may explain the greater overall body condition of urban individuals in this study. For example, higher protein intake is noted to increase immune-defence capabilities in many bird species

(Schmid-Hemple 2003). Furthermore, food availability and nutritional content are tied to immune defence and regulation and can be related to parasite loads (Schmid-Hemple 2003).

Conclusions

In summary, body condition was higher in urban individuals as compared to natural ones, in contrast to the initial prediction. It is possible that the urban individuals in this study had access to better food quality and lower predation risk. However, endoparasite rates, measured as oocyte totals, prevalence, and richness, as well as eosinophil ratios from leukocyte profiles, did not differ significantly between habitats, although a trend of greater eosinophil concentrations was observed in urban animals, suggesting an increased endoparasitism effect. It is likely that an interaction exists between urban and natural individuals in sites chosen for this experiment, and that endoparasite types were transferred between the two habitats. Although I expected this trend to be similar to the trends I noted in body condition and eosinophil concentrations, it is possible that other factors such as stress, food availability, predation risk, and age of infect can influence those differences in ways independent from parasitism rates.

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Tables and figures

Table 2.1. Coordinates of sites used to sample eastern chipmunks (*Tamias striatus*) sampled from Algonquin Provincial Park, Sudbury, and Huntsville Ontario

Site Name	Coordinates	Habitat type
Algonquin park one	45°32'N, 78°36'W	Natural
Algonquin park two	N 45° 32' 40.9065 , W 78° 41' 45.64	Natural
Algonquin park three	N 45° 33' 54.6862 , W 78° 33' 59.6101	Natural
Highway 144	N 46° 36' 30.2195, W 81° 24' 32.0229	Natural
Highway 17	N 46° 25' 27.0767, W 81° 9' 37.7079	Natural
Lake Laurentian conservation area	N 46° 27' 19.6303, W 80° 56' 51.1412	Natural
Huntsville	N 45° 20' 25.4029, W 79° 14' 12.1305	Urban
Huntsville	N 45° 19' 45.5451, W 79° 13' 19.1193	Urban
Huntsville	N 45° 19' 27.7858, W 79° 12' 31.4769	Urban
Laurentian campus	N 46° 28' 5.3785, W 80° 58' 19.4448	Urban
Sudbury 1	N 46° 28' 47.8514, W 81° 0' 25.0784	Urban
Sudbury 2	N 46° 26' 39.7066, W 81° 3' 20.7202	Urban

Sudbury 3	N 46° 28' 2.1214 W 80° 57' 28.7511	Urban
Sudbury 4	N 46° 26' 59.1406, W 80° 59' 16.4343	Urban

Table 2.2. Linear model analysis on the parasite eggs count per gram of wet fecal mass calculated from fecal samples collected from eastern chipmunks (*Tamias striatus*, N = 84) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Linear model analysis was performed using the LM function in R statistical package.

	Estimate	Std.Error	t-value	p-value
Intercept	196.29	505.36	0.39	0.70
Habitat	-27.44	306.22	-0.1	0.93
Reproductive condition	49.43	306.38	0.61	0.87
Sex	38.62	318.27	0.12	0.90
Year	-31.97	406.53	-0.08	0.94

Table 2.3. Linear mixed effect analysis on the total number of parasite egg types calculated from fecal samples collected from eastern chipmunks (*Tamias striatus*, N = 84) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	df	p-value
Intercept	3.81	0.55	6.98	78	**<0.05**
Habitat	0.10	0.33	0.32	78	0.75
Reproductive condition	-0.12	0.33	-0.37	78	0.71
Sex	-0.03	0.34	-0.09	78	0.93
Year	-1.28	0.44	-2.91	78	**<0.05**

Table 2.4. Logistic regression analysis on the parasite oocyte prevalence calculated from fecal samples collected from eastern chipmunks (*Tamias striatus*, N = 84) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	p-value
Intercept	3.85	0.55	6.98	**<0.05**
Habitat	0.05	0.33	0.14	0.89
Reproductive condition	-0.07	0.33	-0.22	0.83
Sex	-0.05	0.34	-0.15	0.88
Year	-1.34	0.43	-3.11	**<0.05**

Table 2.5. Average measurements of skull length (SkL), skull width (SkW), hindfoot length (HFL), and body mass of eastern chipmunks (*Tamias striatus*, N = 133) sampled from urban and natural populations in Algonquin Provincial Park, Sudbury, and Huntsville Ontario in 2012 and 2013

Measurement	N	Mean	SD
SkL(mm) (Urban)	57	39.3	2.00
SkL(mm) (Natural)	76	39.8	2.12
SkW(mm) (Urban)	57	21.4	1.19
SkW(mm) (Natural	76	20.6	1.46
HFL(mm) (Urban)	57	33.7	1.80
HFL(mm) (Natural)	76	33.4	1.94
Mass (Urban)	57	92.9	12.7
Mass (Natural)	76	83.9	15.1

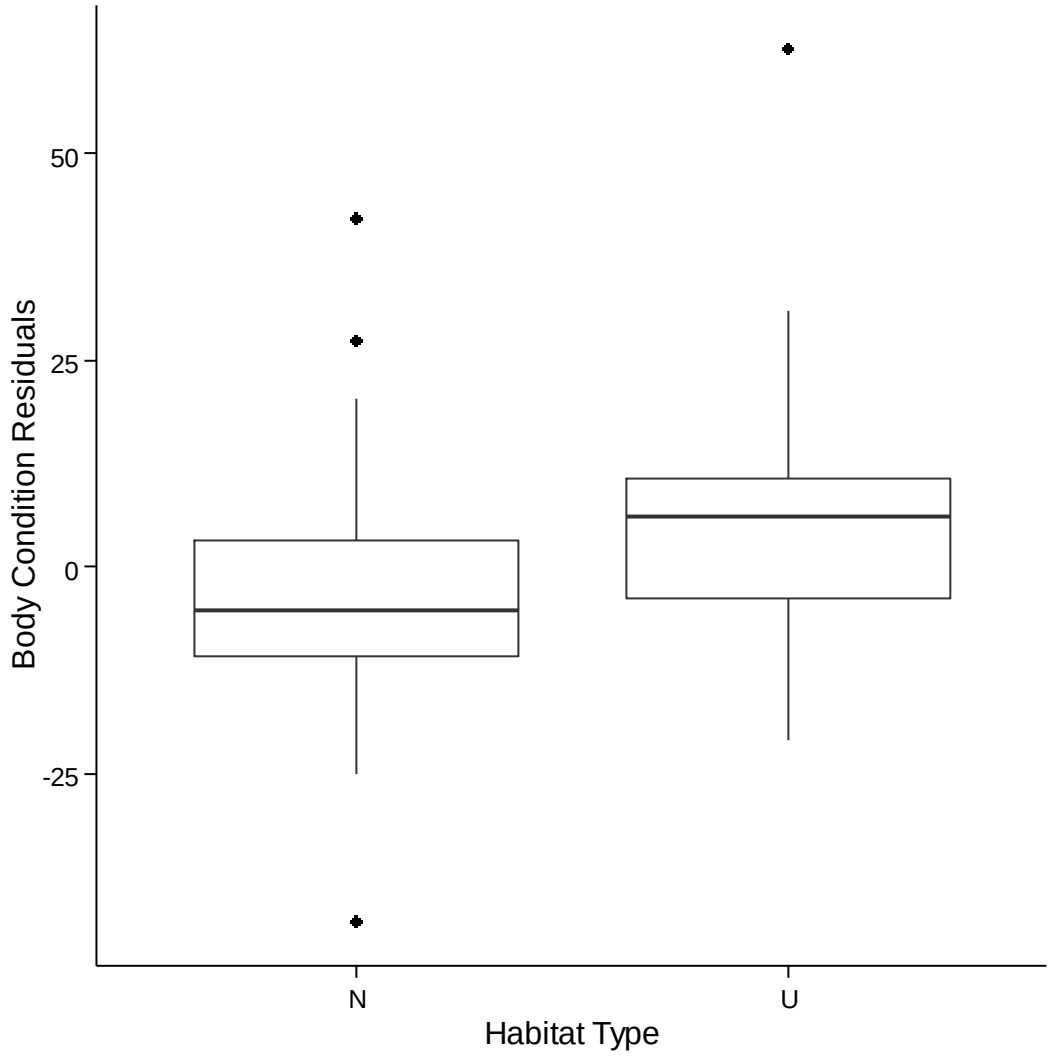
Table 2.6. Linear mixed effect analysis of body condition scores calculated using residual mass, controlling for body size, from measurements collected from eastern chipmunks (*Tamias striatus,* N = 133) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	df	t-value	p-values
Intercept	-2.81	3.91	117	-0.35	**0.00**
Habitat	11.70	3.01	117	3.77	**<0.001**
Reproductive condition	12.40	1.88	117	6.61	**<0.001**
Sex	4.16	2.51	117	1.65	0.10
Year	9.26	2.27	117	3.95	**<0.001**
Habitat*Sex	-7.39	3.81	117	-1.94	**0.05**

Table 2.7. Linear mixed effect analysis on eosinophil percentages in leukocyte samples collected from eastern chipmunks (*Tamias striatus*, N = 90) in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Significant (P<0.05) results are in bold.

	Estimate	Std.Error	t-value	pvalue
Intercept	4.39	1.77	2.49	**0.01**
Habitat	1.53	1.01	1.52	0.13
Reproductive condition	1.36	1.01	1.35	0.18
Sex	0.60	1.02	0.59	0.56
Year	-0.93	1.02	-0.91	0.36

Figure 2.1. Box and whisker plot of the effect of habitat (urban (U)/natural (N)) on body condition scores, from eastern chipmunks (*Tamias striatus*, N = 133, sampled in Algonquin Provincial Park, Sudbury, and Huntsville Ontario. Whiskers represent the first and third quartile -1.5x the IQR, respectively



General Discussion

Summary of results

Rapid expansion and development of human infrastructure expose urban animals to a variety of stressors not encountered in more natural environments (Stockwell *et al.,* 2003, Blumstein *et al.,* 2005, Harris *et al.,* 2013). Human activity and interactions are a primary source of stress to urban animals (Marzluff *et al.,* 2001, Stillman and Goss-Custard 2002, Frid and Dill 2002, Møller 2009, Ashley *et al.,* 2011, Powell *et al.,* 2013). The broad objective of this study was to determine whether body condition, stress, and behavioural syndromes differed between urban and natural populations of eastern chipmunks due to the unique conditions of the former.

The goal for chapter one was to test the hypothesis that differences existed in behavioural syndromes and stress levels between urban and natural habitats. Contrary to my predictions, activity represented by locomotion from behavioural trials was higher in natural animals; however, exploratory behaviour represented by rearing was higher in urban animals as expected. A few scenarios may explain those results: urban animals are accustomed to human exposure and may suffer less from predation in urban settings than do their natural conspecifics, which would support the display of exploratory behaviour after unusual human contact. However, no significant differences were found between cortisol concentrations in feces or in cortisol assessments hair samples, as well as in leukocyte ratios assessed from blood smears. Since forests bordered the urban areas I used, it is possible that dispersal occurs between urban and natural habitats, and this reduces phenotypic differences between the two habitat types

The goal for chapter 2 was to determine if body condition differences were present between animals from urban and natural habitats, and to determine if differences in endoparasite presence, measured in oocyte totals, types present, prevalence, and percentages of eosinophils from leukocyte

counts. Contrary to my predictions, body condition scores were higher in urban animals, suggesting that urban animals have access to greater food quantities or food resources of superior quality. However, parasite load did not differ between the two habitat types. This is unexpected as superior body condition can be correlated with reduced parasite infection. Similarly, no difference was detected in eosinophil percentages between animals from either habitat type. Many endoparasites have been shown not to significantly influence host energy reserves in the short term, suggesting they would not significantly influence body condition. Furthermore, a number of external factors such as endoparasite species interactions, food availability and quality, behavioural modification, as well as timing of infection can influence measurable endoparasitism impacts such as eosinophil percentages and body condition scores.

Future directions

Replicate study using additional areas

Due to the size and location of the urban areas I chose for the study, there is a strong possibility that separation between urban and natural habitats does not occur as strongly as in other potential sites. Both Huntsville and Sudbury are small to medium sized cities, and both are surrounded by expanses of forest. Areas within urban sites that have canopy cover or are otherwise occupied by vegetation provide passageways for wildlife that can connect otherwise isolated habitat fragments (Munshi-South 2012). Though small mammals such as chipmunks generally remain in their own territory (Yerger 1955), some degree of migration does occur, even across roads and other anthropogenic barriers (McGregor *et al.*, 2008). Therefore, given the results there is a strong possibility that interbreeding occurs between the urban and natural populations in my study. Specifically this may explain why many of the criteria I observed did not display significant

differences between the two study habitat types. For example, this may help explain why no differences were detected in behavioural syndromes or oocyte infections. Repeating this study in other cities, particularly in larger, more densely human populated areas where a system of isolated habitat patches occurs within an urban matrix (Munshi-South 2012) may help determine if my findings were the result of urban/natural interaction or if they represent a broader view of chipmunk behaviour. Few studies have looked at multiple species across several different cities (Bonier 2012); a replicate of the study in a larger scale, with more densely populated urban center may yield different results and be warranted for further study.

Replicate study using additional mammal species

Similarly, it would be of interest to repeat this study on other small mammals, since few studies of this kind exist in mammals (Adams 2005, Bonier 2012, Munshi-South 2012). Increasing knowledge of how urbanization impacts species is necessary to learn how negative changes can be reversed (Adams 2005). In particular, comparing traits such as endocrine responses in animals that avoid urban sites with those that do inhabit urban centers may help further studies of potential urbanization impacts in other species (Bonier 2012). Finally, similar studies using birds have shown differing results between multiple bird species living in the same habitats (Bonier 2012), so it would be of interest to see if this also applies to urban mammal populations. Determining how urbanization affects each species is important to help predict urbanization effects on various species in future scenarios.

Dietary preference

The body condition and cortisol analysis suggested that animals sampled from urban habitats were in better health and less stressed than their natural counterparts. Examining the factors that may have influenced observable endoparasite effects, such as diet, may help explain the results I observed, as food availability and nutritional content are tied to immune defence and regulation and can be related to parasite loads and reduced stress (Fokidis *et al*., 2009, Schmid-Hemple 2003). Higher food quality in urban centers as compared to natural areas might also explain the overall greater body condition of urban individuals in my study. For example, higher intake of protein has been noted to decrease cortisol production in urban birds, and is also used as a method to cope with higher rates of parasitism (Fokidis *et al.*, 2009). Similarly, urban animals may have greater access to food resources as a result of consuming human garbage; however, many forms of human garbage have been noted to provide poor nutritional quality for adult animals (Møller 2009). Therefore, it may be worthwhile to examine the intestinal contents of decreased or euthanized chipmunks, or to observe chipmunk caches, to determine if their diet consists of more protein or more nutritious foods in urban centers than in natural ones.

Parasitism

One puzzling aspect was the lack of differences in endoparasitism rates between habitat types, as clear difference in body condition did exist between the two sites. Though some parasite infections do not have a noticeable effect on body condition (Figeurola *et al.,* 1999), it would be useful to identify the endoparasites to species, by examining the gut contents of euthanized or deceased individuals. This would allow accurate identification to species and determine if any of the effects I observed were the result of life history traits of those endoparasites. Another avenue of interest might be to observe ectoparasites loads. Similar to endoparasites, high ectoparasite rates can be energetically costly and can negatively affect growth, reproductive functions, and body condition

scores (Neuhaus 2003, Hillegass *et al.*, 2010, Gooderham and Schulte-Hostedde 2011). Ectoparasites were collected from the sampled chipmunks via a fine toothed comb; however, I was unable to collect sufficient ectoparasite samples to use for analysis for this research project.

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